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Serum Phospholipases A_2 after Aortobifemoral Reconstruction

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Summary: Phospholipase A_2 has been implicated in the pathogenesis of local and distant tissue injury after ischaemia and reperfusion. A common operation inducing ischaemia and reperfusion is aortobifemoral reconstruction, during which the aorta is clamped and the blood supply via the inferior mesenteric artery and iliac arteries is interrupted. The purpose of the present work was to study the catalytic activity concentration of phospholipase A_2 and the mass concentrations of group I and group II phospholipases A_2 in the sera of patients undergoing aortobifemoral reconstruction. Both the catalytic activity concentration of phospholipase A_2 and the mass concentrations of group I and group II phospholipases A_2 increased in serum samples after the operation. The catalytic activity concentration of phospholipase A_2 correlated well with group II phospholipase A_2 mass values ($r = 0.81$, $p < 0.001$), whereas no correlation was found between the catalytic activity concentration of phospholipase A_2 and group I phospholipase A_2 mass values ($r = 0.12$, $p = 0.54$). The results emphasize the role of group II phospholipase A_2 in tissue injury after ischaemia and reperfusion.

Introduction

Phospholipases are hydrolytic enzymes widespread in nature. Phospholipids are the main lipid constituents of all cell membranes and phospholipases control a number of vital cell functions including membrane and cell integrity, signal transduction, secretion, prostaglandin synthesis and cell proliferation (1–5). Phospholipase A_2 is an enzyme of considerable interest with regard to the pathogenesis of various diseases, since its reaction products, lysophospholipids and non-esterified fatty acids, are highly cytotoxic substances (6).

Phospholipase A_2 has been postulated to be involved in the development of tissue injury after ischaemia and reperfusion (7–11). Phospholipase A_2 inhibitors effectively decrease local (7, 12) and distant (10) tissue damage after ischaemia and reperfusion.

Secretory, small molecular mass (M_r 14 000) phospholipases A_2 can be divided into group I phospolipases A_2 and group II phospholipases A_2 based on their amino acid sequences (13). The recent development of specific immunoassays of group I and group II phospholipases A_2 significantly contributed to studies on the role(s) of these enzymes in various diseases. The best characterized function of group I phospholipase A_2 is digestion of the phospholipid component of dietary fat (14), but the enzyme also functions as a growth factor (1) and vasoactive substance (15), and stimulates prostaglandin synthesis (16). Group II phospholipase A_2 is involved in the inflammatory reaction (17), but the details of the role of group II phospholipase A_2 are unknown. The amount of group II phospholipase A_2 is markedly increased in serum during infection and inflammation (17, 18), and the enzyme may function in defence against bacteria (19). Very high levels of circulating group II phospholipase A_2 are associated with sepsis, and a pivotal role was recently postulated for group II phospholipase A_2 in the pathogenesis of circulatory collapse in septic shock (20). The source of group I phos-

pholipase A_2 found in serum is the pancreas, whereas the source of group II phospholipase A_2 is unknown. Further, it is not known which of these secretory phospholipases A_2 is responsible for the increased catalytic activity concentration of phospholipase A_2 in serum after ischaemia and reperfusion. It has been hypothesized that activated pancreatic digestive enzymes such as group I phospholipase A_2 may play an important role in ischaemic intestinal injury by digesting various structures of mucosal cells (21).

A common operation inducing ischaemia and reperfusion in the descending colon and lower extremities is aortobifemoral reconstruction, during which the blood supply via the inferior mesenteric artery and iliac arteries is interrupted. The purpose of the present work was to study the catalytic activity concentration of phospholipase A_2 and the mass concentrations of group I and group II phospholipases A_2 in sera of patients undergoing aortobifemoral reconstruction. Specifically, the questions to be answered were: Does the catalytic activity concentration of phospholipase A_2 change in sera of patients undergoing aortobifemoral reconstruction? Which one of the secretory phospholipases A_2 (group I or II) is involved?

Patients and Methods

Ten consecutive patients undergoing aortobifemoral reconstruction with Y-prosthesis for aortoiliacal occlusive disease (n = 8) or aortic aneurysm (n = 2) in the University Central Hospital of Turku were enrolled in the present study. The mean age of the patients was 55 (range 45–69) years and there were six females and four males. The study was approved by the local Ethical Committee and an informed consent was obtained from each patient.

The Y-prosthesis was anastomosed end-to-end to the aorta below the origins of renal arteries and the distal anastomoses were reconstructed during the same aortic cross-clamp. The inferior mesenteric artery was ligated at its origin from the aorta in patients with aortic aneurysm, whereas the blood flow to the inferior mesenteric artery was established at reperfusion via the stenotic iliac arteries in patients operated for arteriosclerotic disease.

The operations were performed under general anaesthesia with thiopentone, pancuronium, fentanyl, dehydrobenzperidol, enflurane (volume fraction 0.005–0.01) and nitrous oxide (volume fraction 0.70) plus oxygen (volume fraction 0.30). The patients had a catheter inserted into a radial artery for haemodynamic monitoring. End-tidal CO_2 was monitored and kept at a level of normoventilation (4.5–5.5 kPa). Blood loss was replaced with *Ringer*'s acetate solution and with packed red blood cells, when necessary, to maintain the blood haemoglobin concentration above 100 g/l. After the operation, atropine and neostigmine were used to reverse the neuromuscular blockade and the patients were extubated in the operating room. The mean blood loss was 1400 (range 100–5800) ml, operation time 161 (125–185) min, aortic clamping time 61 (39–106) min and the deepest fall in blood pressure after aortic declamping 37 (10–75) mmHg. All the patients recovered uneventfully.

Blood samples were withdrawn before the operation, before aortic clamping, after declamping, three and six hours after declamping, and thereafter daily until the eighth postoperative day to determine the catalytic activity concentration of phospholipase A_2 and the mass concentrations of group I and group II phospholipases A_2 in serum. The catalytic activity concentration of phospholipase A_2 was assayed by using micellar radioactive phosphatidylcholine as a substrate (22). The mass concentrations of group I and group II phospholipases A_2 were measured by time-resolved fluoroimmunoassays as described earlier (23, 24). The serum samples were stored at −20 °C until assayed.

The results are expressed as mean ± SEM. The significance of the time-related changes was tested by the analysis of variance (ANOVA) with repeated measurements. *Newman-Keuls*' multiple range test was used to test differences between the preoperative and other values. *Pearson*'s linear correlation was used to study the correlations between the catalytic activity concentration of

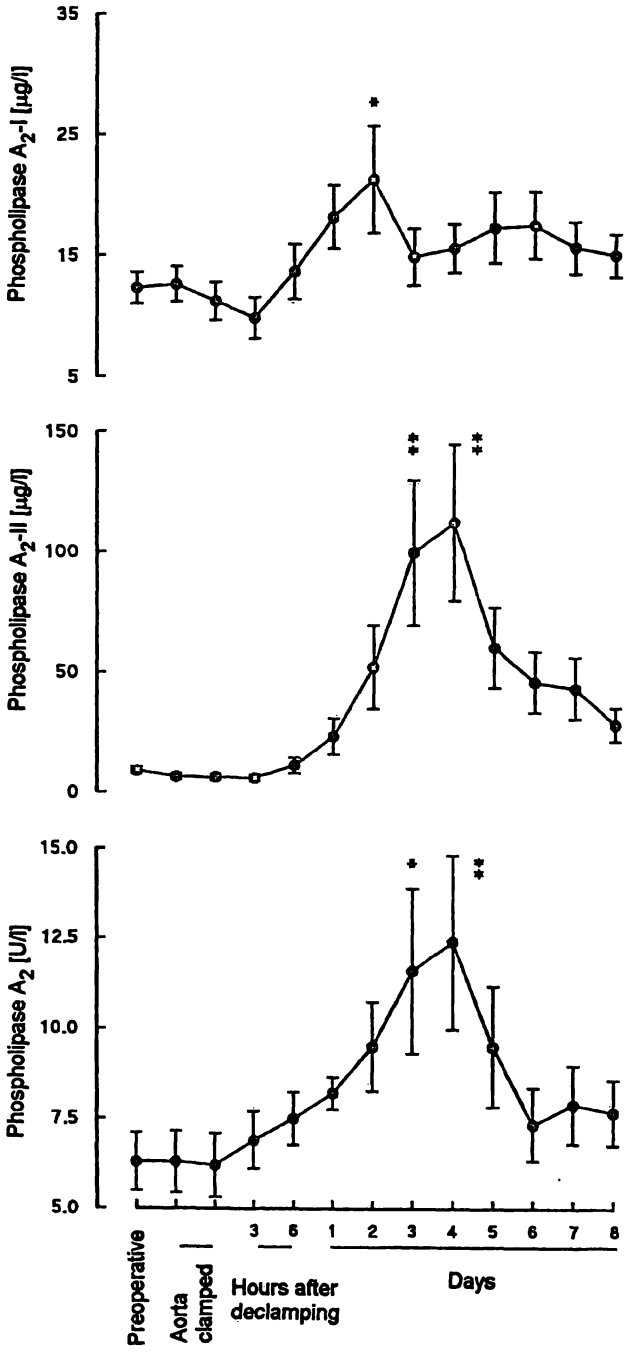

Fig. 1 Group I phospholipase A_2, group II phospholipase A_2 mass concentration and the catalytic activity concentration of phospholipase A_2 in sera of ten consecutive patients undergoing aortobifemoral reconstruction (mean ± SEM). * $p < 0.05$ and ** $p \leq 0.01$ compared with the preoperative values (*Newman-Keuls*' multiple range test).

phospholipase A_2 and the mass concentrations of group I and group II phospholipase A_2.

Results

Figure 1 shows the mass concentrations of group I and group II phospholipases A_2 and the catalytic activity concentration of phospholipase A_2 in sera of the operated patients. The mass concentrations of group I and group II phospholipases A_2 and the catalytic activity concentration of phospholipase A_2 increased steadily during days 1–4 after the operation and decreased thereafter ($p < 0.001$). The mass concentration of group II phospholipase A_2 and the catalytic activity concentration of phospholipase A_2 changed in concert and reached in their peak values on the fourth postoperative day, while the mass concentration of group I phospholipase A_2 reached its maximum at the second postoperative day. The patients did not show any clinical signs of postoperative pancreatitis.

The catalytic activity concentration of phospholipase A_2 correlated well with the mass concentration of group II phospholipase A_2 in the regression analysis (fig. 2; $r = 0.81$, $p < 0.001$), whereas there was no correlation between the catalytic activity concentration of phospholipase A_2 and the mass concentration of group I phospholipase A_2 (fig. 3; $r = 0.12$, $p = 0.54$).

Discussion

Increased phospholipase A_2 activity seems to be instrumental for the development of ischaemic intestinal injury (11). Phospholipase A_2 inhibitors effectively decrease tissue damage and the increase in mucosal permeability in experimental intestinal ischaemia (7, 12). Moreover, increase of phospholipase A_2 activity is regarded a crucial step in the pathogenesis of distant organ injury after ischaemia and reperfusion, since phospholipase A_2 inhibition prevented lung injury after ischaemia and reperfusion of the gut (10). Phospholipase A_2 is involved in the generation of a variety of bioactive and potentially toxic lipid metabolites, such as arachidonic acid metabolites, lysophospholipids, and platelet-activating factor (6), which may play a role in local and distant tissue injury after ischaemia and reperfusion. In addition, damage at the mucosal layer of the intestine allows enhanced uptake of bacteria and endotoxins from the intestinal lumen into circulation (25), which, in turn, increases the formation of inflammatory mediators such as phospholipase A_2 leading to a decrease in blood pressure and circulation (20). These changes may create a vicious circle in which impaired intestinal perfusion leads to a progressive damage of intestinal mucosal cells resulting ultimately in bowel infarction. In aortobifemoral reconstruction, the blood supply from the inferior mesenteric artery is interrupted, and, thus, this operation always induces left colonic ischaemia to some extent. Although the resulting ischaemic colonic injury usually runs a benign course, a fulminant disease develops in a minority of patients. This may prove fatal in spite of prompt surgical intervention. The results of the current study emphasize the role of group II phospholipase A_2 in tissue injury after ischaemia and reperfusion.

The mass concentration of group I phospholipase A_2 increased in sera of patients after aortobifemoral reconstruction in the current study, but no clinical signs of postoperative pancreatitis were noticed. The slight increase in serum group I phospholipase A_2 mass concentration may be due to pancreatic irritation during the operation. Group I phospholipase A_2 mass concentration values did not correlate with the catalytic activity con-

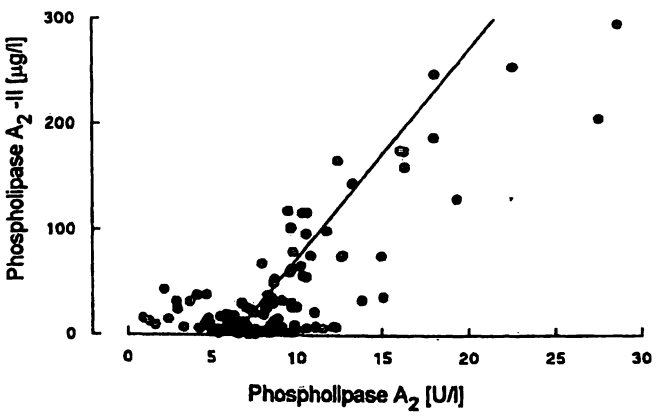

Fig. 2 Linear regression between the catalytic activity concentration of phospholipase A_2 and the mass concentration of group II phospholipase A_2 in sera of patients undergoing aortobifemoral reconstruction ($r = 0.81$, $p < 0.001$).

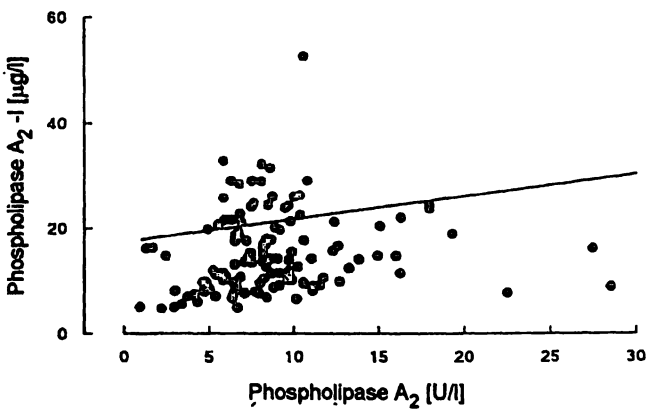

Fig. 3 Linear regression between the catalytic activity concentration of phospholipase A_2 and the mass concentration of group I phospholipase A_2 in sera of patients undergoing aortobifemoral reconstruction ($r = 0.12$, $p = 0.54$).

centration of phospholipase A_2. The results of the current study do not support the formerly presented idea (21) that activated/increased pancreatic digestive enzymes such as group I phospholipase A_2 may play an important role in ischaemic intestinal injury.

The current results show that the catalytic activity concentration of phospholipase A_2 and the mass concentrations of group I and group II phospholipases A_2 increase in sera of patients after aortobifemoral reconstruction. The catalytic activity concentration of phospholipase A_2 correlates well with the mass concentration of group II phospholipase A_2 but not with the mass concentration of group I phospholipase A_2 in sera of these patients. These results indicate that the increase in the catalytic activity concentration of phospholipase A_2 is due to group II phospholipase A_2. We conclude that group II phospholipase A_2 may be involved in the development of local and distant tissue injury after ischaemia and reperfusion.

Acknowledgements

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